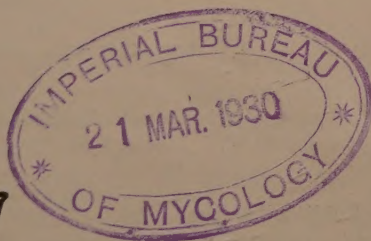
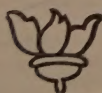


BACTERIAL BLIGHT OF POPPY CAUSED BY BACTERIUM PAPAVERICOLA, SP. NOV.

BY

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(Contribution from Bureau of Plant Industry)

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BACTERIAL BLIGHT OF POPPY CAUSED BY BACTERIUM PAPAVERICOLA, SP. NOV.¹

By MARY K. BRYAN, Associate Pathologist, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and FRANK P. McWHORTER, Pathologist, Virginia Truck Experiment Station

INTRODUCTION

A striking disease of the Shirley poppy (*Papaver rhoeas* L.) was observed by the junior writer while visiting the Council plant farm, Franklin, Va., in May, 1927. Since the disease was decidedly destructive, probably of bacterial etiology, and apparently unlike any heretofore-described poppy disease, a rather full description of it was immediately sent to the Office of Mycology and Disease Survey of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C. The present paper is a preliminary report intended to establish the bacterial nature of the disease and to describe the pathogene as a new species of Bacterium.

HISTORY OF THE DISEASE IN THE TYPE LOCALITY

Through visits to the garden of the Council plant farm, Franklin, Va., and by correspondence with its owner, the writers are able to present the following brief history of the garden plot from which the type material was collected.

Seeds of the Shirley poppy were planted in the spring of 1926. According to the grower, a few of the plants developed the disease during the latter part of the first (1926) season; at that time the disease was destructive to the individual infected plants, but not to the plot as a whole. The 1926 plants reseeded the area and served as a source for the 1927 crop. The 1927 plants, when small, were given a generous application of sheep manure, which, according to the grower, may have predisposed the plants to the disease, for during the 1927 season it was conspicuous even before the plants reached the flower-bud stage. By the middle of the blooming season many of the plants were killed, and those remaining were made unsightly by numerous black spots on leaves and buds. While the plants were in this condition the owner burned over the area, hoping to eradicate the disease and to prevent the poppies from reseeding. Some escaped, however, for in 1928 a few came up; these were left for a while, but since they developed the disease long before blooming time, they too were burned.

Type material was collected from this plot by the writers in May, 1927, and later filed in the herbarium of the Office of Mycology and Disease Survey.

¹ Received for publication Apr. 29, 1929; issued January, 1930. This paper resulted from a joint project carried out by the Virginia Truck Experiment Station, Norfolk, Va., and the Bureau of Plant Industry, U. S. Department of Agriculture.

IMPORTANCE AND GEOGRAPHICAL DISTRIBUTION

The behavior of this disease on poppies at Franklin, Va., points to its having great possible economic importance. It not only made the plants unsightly by conspicuous black spotting of the leaves and stems but also killed many of them. Moreover, later infection tests have shown that it is readily transmissible.

The writers have observed this disease on the Shirley variety at the Franklin locality only. That it has occurred elsewhere in the United States is shown by a report of Clinton² in 1909 and by herbarium material kindly furnished by him. Clinton's collection of the leaf spot form of the disease was made at Westville, Conn. In appearance, the lesions are identical with those on the Virginia specimens. Bacteria were found in great abundance in the spots, but, as was expected, attempts to isolate them from this herbarium material failed.

In addition to the Shirley poppy, the Oriental poppy (*Papaver orientale* L.) must be considered as susceptible. Mature pods and leaves of Oriental poppies with lesions similar to those on Shirley poppy were collected at North, Va., by the junior writer in June, 1927. The organism isolated from these leaves showed slight cultural variations from the Shirley poppy strain, but when inoculated into Shirley poppies it produced lesions characteristic of the disease.

SYMPTOMATOLOGY

The following discussion is based on the examination of plants naturally infected in the type locality and on observation of plants subsequently inoculated with pure cultures of the organism. Lesions were abundant on all the aerial parts, but for convenience in discussion the symptoms on leaf, stem, and floral parts are presented separately.

EFFECT ON LEAVES

The general appearance of infected leaves is shown in Figures 1 and 2. The intensely black spots suggest those produced on rose leaves by *Diplocarpon rosae* Wolf.

Young infections are first evident as minute water-soaked areas; these soon darken into definite spots bounded by a hyaline or water-soaked ring. This ring is seldom evident in older spots.

The size and the shape of mature spots are affected by the distribution of the primary lesions on the leaf. When scattered they are distinctly circular, clearly defined, often zonate (fig. 3), and may attain a diameter of 3 to 4 mm. Eventually the tissues between the spots yellow and turn brown, but the individual spots remain distinguishable by their darker color and zonate markings. The zonation is comparable to that developed in wildfire of tobacco and bacterial leaf spot of *Delphinium*. When the infections occur close together, the individual spots are small (1 mm. or less in diameter), but by coalescing they form large black or sepia-black areas. A slimy bacterial exudate is noticeable at times on the lesions. Leaf infections weaken the leaves to such an extent that defoliation results.

² CLINTON, G. P. REPORT OF THE BOTANIST. Conn. Agr. Expt. Sta. Bien. Rpt. 1907-08 (pt. 12): 870, 1909.

Entrance of the bacteria is accomplished through the stomata. (Pl. 1, B.) Sometimes the organism enters the veins from the intercellular spaces or through the water pores at the tips of the serratures, so that the disease becomes systemic. When this occurs the veins darken and the surrounding tissues yellow and deaden, thereby assuming the appearance shown in Figure 4. This effect suggests the behavior and appearance of water-pore lesions typical of black rot on cabbage, but cross inoculations between *Bacterium campestre* and the poppy organism were negative.

EFFECT ON THE STEMS

The lesions on the stems are intensely black, as are those on the leaves (fig. 5); but, unlike the leaf lesions, the stem lesions tend to elongate longitudinally. When close together they may coalesce and girdle the stems. They are confined chiefly to the cortical regions, but the girdling process is sufficiently severe to cause the smaller plants to break over.

EFFECT ON FLORAL PARTS AND PODS

All floral parts are susceptible, but the young sepals are especially so; these may be partially or entirely blackened. (Fig. 5.) The organism is able to pass through the sepals and attack the young petals beneath. When this occurs further development of the flowers is stopped.

The spots on the pods (fig. 5), like those on the leaves, are black and conspicuous, but the margins are more promi-



FIGURE 1.—Shirley poppy leaf showing isolated spots of infection. About twice natural size

nently water-soaked. The slimy appearance of the spots, referred to above, is more noticeable on the pods than on any other part of the plant.



FIGURE 2.—Shirley poppy leaf with numerous scattered spots of infection and large dead areas formed by coalescing spots. Natural size

GENERAL EFFECT ON THE PLANT

The general effect on the plants has a twofold economic significance. In the first place, the conspicuous black leaf spots make the plants unsightly; and secondly, many of the plants are killed. The killing is caused in part by the girdling of the stems, in part by defoliation, and in part by the toxic or mechanical effect produced by masses of the organism when the disease becomes systemic.

PREVIOUSLY REPORTED BACTERIAL DISEASES OF POPPY

The only report of bacterial blight of poppy, previous to that made by the junior writer, is that of Clinton.³ Other bacterial diseases of poppy have been reported as noted below.

Marchal and Foex⁴ in 1915 described a blight of the sepals and petals of poppy, but they did not designate the species from which they isolated a white bacterium similar to *Bacillus coli communis*. They did not report any inoculations with the organism that they isolated. The organism described in the present paper produces a yellow pigment.

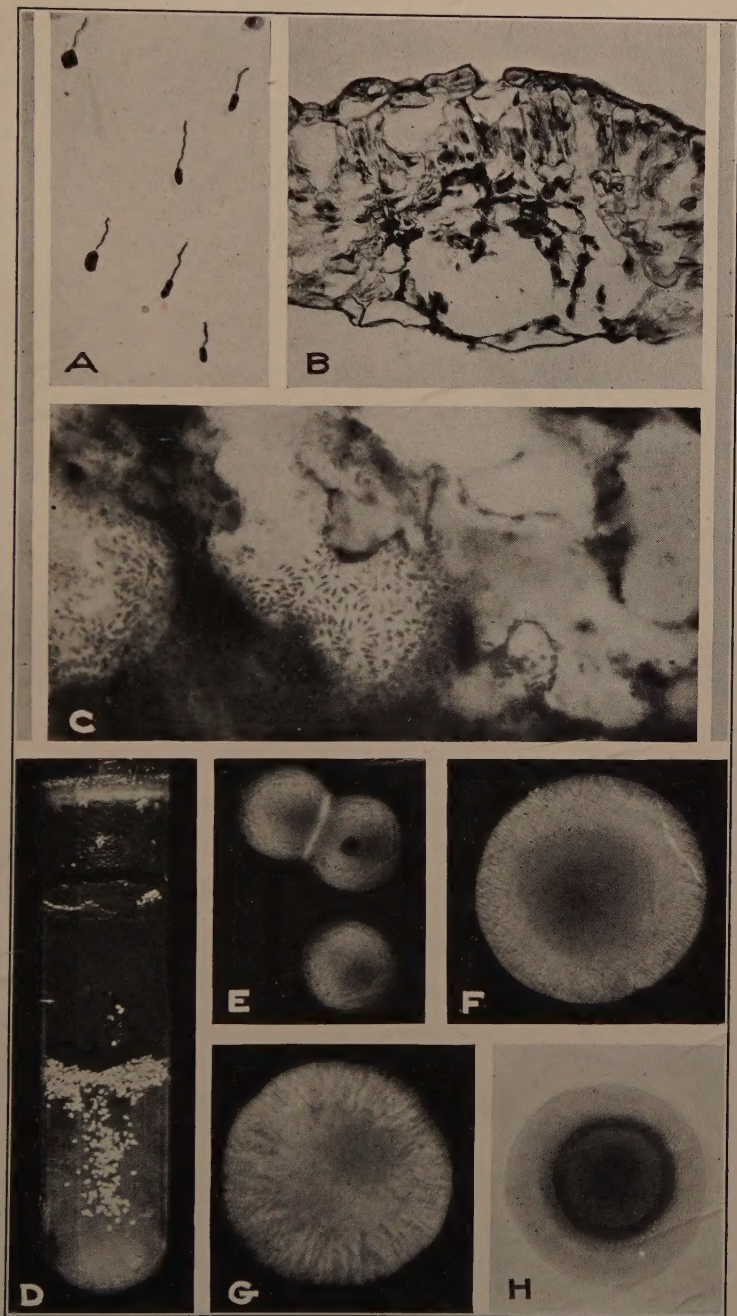
In 1918 Hutchinson⁵ reported from India a poppy disease which he considered to be due to bacteria. In describing it he stated:

"Blackening and slimy decay of the stem and leaves of the opium poppy and other varieties was found to be due to bacterial

³ CLINTON, G. P. Op. cit.

⁴ [MARCHAL, P., and FOEX, E.] RAPPORT PHYTOPATHOLOGIQUE POUR L'ANNÉE 1913. Ann. Serv. Epiphyties 2:63-64. 1915.

⁵ HUTCHINSON, C. M. AGRICULTURAL BACTERIOLOGY. India Bd. Sci. Advice Ann. Rpt. 1916-17: 57-66. 1918.



A.—*Bacterium papavericola* showing flagella. $\times$ about 1,100.

B.—Section of inoculated leaf showing stomatal infection. $\times$ 200.

C.—Section of diseased leaf showing bacteria in the tissues. $\times$ 1,150.

D.—Milk culture 1 month old, showing masses of tyrosin crystals.

E-H.—Colonies on beef-infusion agar plates. $\times$ 5. Colony E, 3 days old; colonies F, G, and H, 5 days old. Colonies E, F, and G by oblique transmitted light; colony H by direct transmitted light.

rot; a description of the disease and of the causative organism is in hand for publication." It appears that Hutchinson did not publish his data but instead turned them over to Ram Ayyar,⁶ as reported below.

In 1927 Ram Ayyar⁷ described from Pusa, India, a bacterial soft rot of garden poppy which is apparently that referred to by Hutchinson. The disease and the causal organism described by him are different from those recorded in this paper. The Indian disease found on *Papaver somniferum* at Cawnpore by Hutchinson later developed on *P. rhoeas* at Pusa, and the latter species was used in inoculation experiments by Ram Ayyar. Ram Ayyar made no reference to leaf spotting, but instead he described the disease as a soft rot of the stem and the organism as a peritrichiate, rod-forming, white or bluish-white growth on various culture media. The bacterial-blight organism of the present paper forms a yellow pigment and produces no soft rot.

ISOLATIONS AND INOCULATIONS

The organism was found abundantly in old and young lesions on both species of the poppies studied. (Pl. 1, C.) It was readily isolated from fresh material by the poured-plate method, and subcultures from single colonies were used in inoculation experiments.

On June 20, 1927, a group of Shirley poppy plants in a garden, the only ones then available, were sprayed with a suspension of the pathogene isolated on June 2 from Shirley poppy. A gentle rain which continued during the evening provided sufficient moisture so that it was not necessary to cover the plants with inoculation cages. Infections were evident on stems and leaves on June 25.

Pure-culture reisolations made on June 28 from spotted leaves on these inoculated plants were used on September 21 to inoculate plants of Shirley and Oriental poppies by spraying. These plants were kept moist in inoculation cages in the hothouse for 48 hours. The Shirley poppies used were blooming plants, whereas the Oriental

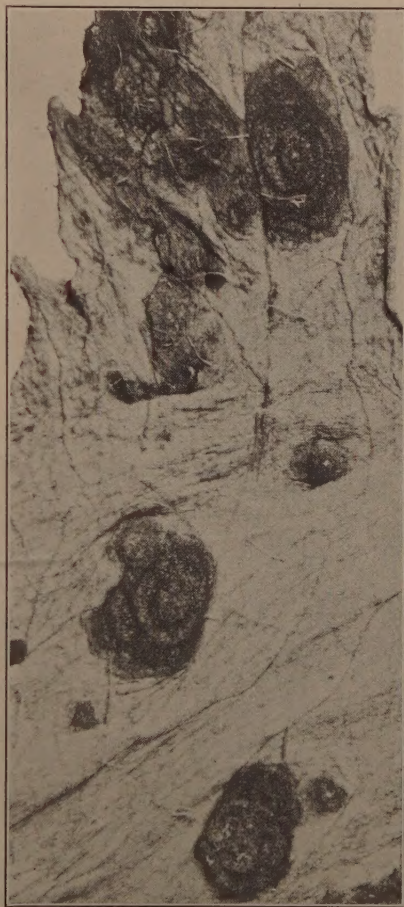


FIGURE 3.—Isolated spots of infection on Shirley poppy leaf, enlarged to show zonate character. $\times 5$

Infections were evident on stems

⁶ RAM AYYAR, C. S. A BACTERIAL SOFT ROT OF GARDEN POPPY. India Dept. Agr. Mem., Bact. Ser. 12: 29-33, illus. 1927.

⁷ RAM AYYAR, C. S. Op. cit.

ones were seedlings with only a rosette of leaves. All were vigorous. Successful infections were thus obtained on the leaves, buds, and seed pods of the Shirley poppy and on the leaves of the Oriental poppy. On October 7 the pathogene was reisolated from all parts of these infected plants.

Poured-plate isolations from spots on Oriental poppy leaves collected at North, Va., were also used on September 21 to inoculate both species of poppy. The same method was used as with the isolations from the Shirley poppy. Infections resulted on both species, and successful reisolations were made.

Good infections were also obtained when Shirley poppy plants in an outdoor plot were sprayed with the pathogene and kept moist under large flower pots.

The Shirley poppy appears to be more susceptible than the Oriental poppy, judging by the number of infections resulting from these series of inoculations.

THE PATHOGENE

MORPHOLOGY

Bacterium papavericola, sp. nov., is a short rod, 1 to 1.7μ long by 0.6 to 0.7μ wide, motile by means of a single polar flagellum. (Pl. 1, A.) It occurs singly, in pairs, or in short chains: Capsules occur in old agar cultures and in the rims and pellicles of bouillon cultures. No spores have been observed.

STAINING REACTIONS

The organism is decidedly Gram-negative and is not acid-fast. It stains readily with the usual bacterial stains.

CULTURAL CHARACTERS

Unless otherwise stated, the descriptions here given are based on cultures of the Shirley strain, but they are true also for those of the Oriental strain. Slight differences in amount rather than character of growth have been disregarded.

BEEF-AGAR PLATES.⁸—Colonies are visible on the second day, and by the third day they are 2 to 3 mm. wide, mustard yellow,⁹ smooth, convex, round, finely crosshatched by transmitted light, translucent, margin entire. (Pl. 1, E.) Radiating lines are sometimes conspicuous (pl. 1, G) and at other times entirely lacking. (Pl. 1, F.) Colonies may attain a width of 7 mm. and often are slightly umbonate, the thinner margin showing as a paler yellow ring by transmitted light. (Pl. 1, H.) Secondary growths of paler color, in the form of

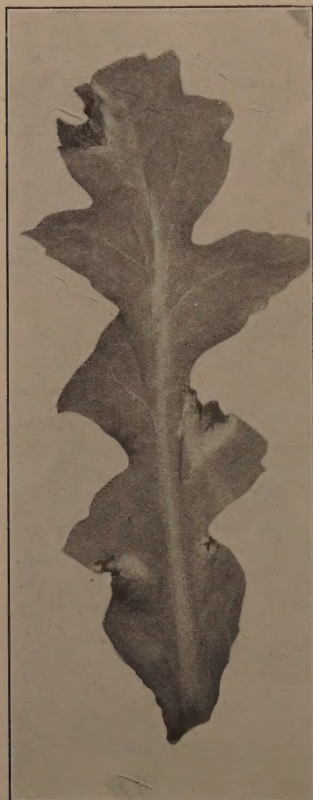


FIGURE 4.—Systemic infection on leaf of Shirley poppy. Natural size

⁸ Unless otherwise noted, all beef media used were made with beef infusion and peptone and had a pH of 6.8 to 7.0.

⁹ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

wedges on the margin or circular areas in the body of the colony, are common in old colonies. A second type of colony sometimes appears; these are smaller, thinner, and deeper yellow. Colonies of the Oriental strain differ from those of the Shirley strain in internal markings only. They occasionally show fine concentric lines in the margins, but they are often finely granular throughout without other markings.

BEEF-AGAR SLANTS.—Growth is filiform, and scanty at first, becoming moderate but never heavy; it is translucent, smooth, finely granular or crosshatched, butyrous in young cultures, becoming viscid when old. The color, at first mustard yellow,¹⁰ becomes primuline yellow with age (2 weeks). Numerous small, circular, secondary growths of paler yellow appear in old cultures.

The growth on slant cultures of the Oriental strain is consistently deeper yellow (primuline to light cadmium) than on those of the Shirley strain.

BEEF BROTH.—Clouding is prompt, and by the second day a pale-yellow rim and often an incomplete pellicle floating unattached to the rim have formed. In undisturbed cultures the pellicle becomes heavy and may or may not connect with the rim. Numerous small rectangular crystals form in the pellicle.

Growth on beef-extract agar and broth does not differ from that on agar and bouillon made with beef infusion.

POTATO CYLINDERS.—Growth is heavy on potato cylinders, covering the whole surface of the potato and filling the water with a dense, yellow slime. The diastatic action is so strong that the cylinder gradually shrinks until almost completely submerged in the yellow bacterial growth, which may eventually take on a brown tinge. Minute brown, spherical aggregates of crystals form in abundance between the growth and the side of the tube in old cultures. Cultures of the Oriental strain are indistinguishable at first from those of the Shirley strain. Eventually the growth becomes a decidedly deeper yellow and less abundant than in the Shirley strain, and the diastatic action is less vigorous.

THAXTER'S POTATO-DEXTROSE AGAR.—Growth on slant cultures is very abundant, pale yellow, and homogeneous. Color deepens with age to primuline yellow. The Oriental strain remains paler than the Shirley one on this medium.

SYNTHETIC MEDIA.—In Cohn's solution growth may not occur, and at best it is almost imperceptible. Fermi's solution clouds weakly but develops no fluorescence. In Uschinsky's solution clouding is slow, becoming moderate but without fluorescence.

PHYSIOLOGICAL CHARACTERS

LIQUEFACTION OF GELATIN.—In beef-infusion gelatin held at 17° to 20° C. liquefaction begins in two or three days; it is saucer shaped at first, but later it becomes stratiform. It proceeds so slowly that after two weeks only one-half

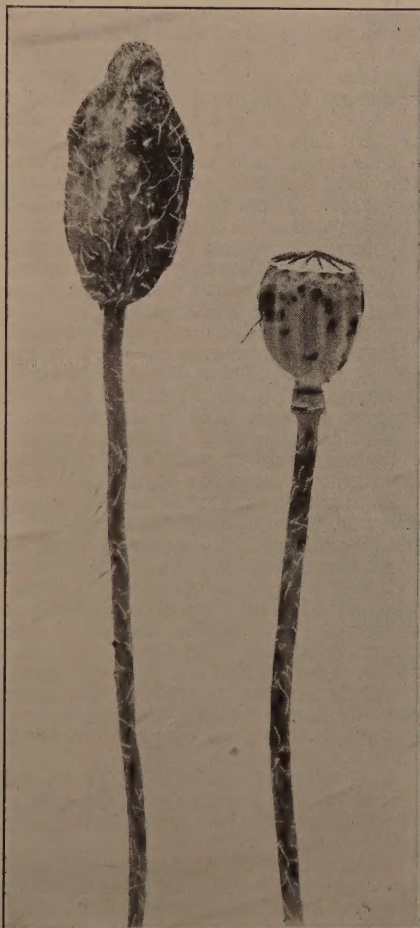


FIGURE 5.—Infections on bud, pod, and floral stalk of Shirley poppy. Natural size

¹⁰ RIDGWAY, R. Op. cit.

is liquefied. Liquefaction is not complete even when the cultures are held for six weeks. The liquefied portion is clear, with a yellow pellicle and abundant sediment.

LIQUEFACTION OF BLOOD SERUM.—Liquefaction, which is preceded by clearing of the medium, begins by the third day; clearing and liquefaction proceed so slowly that in three weeks only one-half of the medium is liquefied.

FERMENTATION OF SUGARS.—Various sugars and alcohols were tested for fermentation. On beef-extract agar slant cultures with bromocresol purple as an indicator, acid was evident on the third day from saccharose, dextrose, and galactose. The yellow color progressed slowly to the bottom of the tubes. On the fourth day an alkaline reaction appeared in the upper end of all these cultures, and by the fifteenth day the agar was alkaline throughout in all. At no time was there any evidence of acid formation from levulose, lactose, maltose, glycerin, or mannit in this agar. When phenol red was used as an indicator in the same agar and with the same sugars, an alkaline reaction was evident on the second or third day, and in 10 days the whole of each culture was alkaline.

The organism was tested on the same carbohydrates in synthetic agar made according to the formula in the Manual of Methods¹¹ with bromocresol purple as indicator. There was no trace of alkali at any time in these cultures. An acid reaction was evident in saccharose and dextrose on the second day, on the fifth day in glycerin, and on the third or fourth day in the others. Within three weeks all the cultures were acid throughout.

No gas was formed from these sugars and alcohols in fermentation-tube cultures containing 1 per cent peptone and 2 per cent of the sugar. Clouding was confined to the open ends of the tubes.

HYDROLYSIS OF STARCH.—The starch of steamed-potato cylinders is so completely destroyed that the shrunken cylinders are almost or completely submerged in the mass of bacterial slime. On starch-agar plates streaked with the organism, an area 25 mm. wide is cleared beyond the growth within seven days. The diastatic action of the Oriental strain is decidedly less vigorous. The potato cylinders are never completely submerged. On starch-agar plates the clear area after growth for seven days is only 5 to 8 mm. wide.

REDUCTION OF NITRATE.—No nitrate reduction was evident in nitrate-broth cultures, when 4, 7, and 10 days old, by using the starch-potassium-iodide sulphuric-acid test. However, a weak but decided nitrite reaction was obtained at all these ages by the α -naphthalamine sulphanilic-acid test.

REACTIONS IN MILK.—Separation begins on the second day with a layer of whey 1 to 3 mm. deep above a fluid curd. In litmus milk there is a slight reddening on the third day, followed immediately by reduction of the litmus, which is completed in 4 to 10 days. Color begins to return 3 or 4 days later. Peptonization is evident on the third or fourth day; clearing proceeds slowly downward in bands and is completed in two weeks. In cultures 1 month old the curd is a coarsely flocculent, fluid, translucent mass occupying the lower half of the culture, with a clear whey above, holding numerous masses of tyrosine crystals. (Fig. 4.)

Reduction of methylene blue in milk begins on the third day and is completed on the fourth day. Coagulation, peptonization, and the formation of tyrosine crystals proceed as in litmus milk. Old cultures (1 month) are orange buff in color.

PRODUCTION OF AMMONIA.—Ammonia production is strong, as indicated by tests made with strips of filter paper wet with Nessler's solution and inserted in beef-broth cultures 8 and 10 days old.

PRODUCTION OF HYDROGEN SULPHIDE.—Lead-acetate paper tests for the presence of hydrogen sulphide in beef broth gave on the third day a distinct positive reaction which became strong by the sixth day.

PRODUCTION OF INDOL.—No indol was formed in Dunham's solution within 15 days. *Bacillus coli communis* used as a check gave the usual positive result.

TOLERATION OF SODIUM CHLORIDE.—The organism grows readily in beef broth containing 1.2 or 3 per cent sodium chloride. Growth is retarded by 4 per cent and inhibited by 5 per cent.

RELATION TO FREE OXYGEN.—In agar stabs the best growth occurs at and near the surface; no growth occurs in the bottom of the stabs, or in stabs which have been covered immediately by 10 c. c. of agar. There is no growth in the lower part of shake agar cultures. Growth is confined to the open end of fermentation tubes.

¹¹ SOCIETY OF AMERICAN BACTERIOLOGISTS. COMMITTEE ON BACTERIOLOGICAL TECHNIQUE. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA, FOR USE WITH THE DESCRIPTIVE CHART OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS. 48 p., illus. Geneva, N. Y. [1923.]

OPTIMUM pH FOR GROWTH.—In a series of beef broths ranging from pH 4.4 to 9.6, clouding occurred within 24 hours in pH 6.1 to 7.7, but it was heaviest between pH 6.9 and 7.6. By the second day all were clouded between pH 5.6 and 8.3. No growth took place beyond these limits.

RESISTANCE TO DESICCATION.—Sterile cover glasses wet with a loop from 24-hour-old cultures were placed in sterile Petri dishes and kept dry in the dark. At intervals covers were dropped into beef broth. After drying for four months and at intervals before, the bacteria clouded the bouillon promptly.

In herbarium specimens the bacteria were alive after drying for 20 months. On plates from these dry leaves colonies came up promptly and appeared as vigorous as those from fresh material; they were proved infectious by inoculation on Shirley poppy leaves.

TEMPERATURE RELATIONS.—The thermal death point is 52° C. Best growth occurs between 25° and 30°. The maximum temperature for growth is about 35°.

TECHNICAL DESCRIPTION

***Bacterium papavericola*, sp. nov.**

A short rod, 1 to 1.7 μ long by 0.6 to 0.7 μ wide, motile by means of a single polar flagellum, occurring singly, in pairs, or in short chains. It is capsulate but forms no spores; is Gram-negative and not acid-fast; forms round, bright-yellow colonies on beef agar; clouds bouillon and forms a rim and a pellicle; diastatic action strong; liquefies gelatin rather slowly; forms acid without gas from dextrose, saccharose, galactose, levulose, lactose, maltose, glycerin, and mannit; reduces nitrates; causes weak coagulation and complete peptonization in litmus milk with reduction of litmus and the formation of tyrosine crystals; produces ammonia and hydrogen sulphide but no indol; is aerobic; grows best in a pH of 6.9 to 7.6; withstands drying for 4 months on cover glasses and for 20 months or longer in herbarium specimens; optimum temperature is between 25° and 30° C.; causes black spots on all aboveground parts of the Shirley poppy and on leaves and pods of the Oriental poppy.

SUMMARY

Bacterial blight of poppy is a disease which attacks Shirley and Oriental poppies. It produces unsightly black spots on leaves, stems, buds, and seed pods; it spreads rapidly and when severe may kill the plants.

The disease has been found in Virginia and Connecticut. The organism causing it has been isolated and successfully inoculated into Shirley and Oriental poppies. *Bacterium papavericola* is presented as the name for the pathogene. A description of the cultural and physiological behavior of *Bact. papavericola* is given.



